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Vascularized pulp regeneration via injecting simvastatin functionalized GelMA cryogel microspheres loaded with stem cells from human exfoliated deciduous teeth

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**Article Information**

Xiaojing Yuan, Zuoying Yuan, Yuanyuan Wang et al. designed and conducted an experiment to see if the complex made from stem cells from human exfoliated deciduous teeth (SHEDs) and Simavastin (SIM) was able to induce vascularized pulp regeneration. The study took place in Beijing, China and was published in *Materials Bio Today 13* titled *Vascularized pulp regeneration via injecting simvastatin functionalized GelMA cryogel microspheres laoded with stem cells from human exfoliated deciduous teeth* in January 2022. The authors stated no conflicting financial or competing interests and acknowledged financial support from Natural Science Foundation of China, Beijing Natural Science Foundation, National State Key Laboratory of Oral Diseases, and Stomatology Development Fund of Tason ([https://pubmed.ncbi.nlm.nih.gov/35198958/](https://urldefense.com/v3/__https%3A/pubmed.ncbi.nlm.nih.gov/35198958/__;!!PJt-7SellYIzVQ8!u8H5naiydguTNQZ9C6Az4YPt4jzi__sVY9srlMnbg6XOfFboOU8t-HrMJwBqp_s5UQaCOA$) – <https://doi.org/10.1016/j.mtbio.2022.100209>).

**Summary of Article**

 Specific reagents/solvents were gathered, and different amounts were put into SHEDs culture to see odontogenic and proangiogenic effects on SHEDs cultures. After determining the most effective amount of reagents/solvent, the complex was injected into nude mice to see how findings in vitro compared to findings in vivo. The authors concluded SHEDs with SMS (SIM functionalized cryogel microspheres) showed odontogenic and proangiogenic potential in vitro and regenerated vascularized pulp-like tissue in vivo. This can provide a new outlook and change the methods used in endodontic regenerative dentistry.

**Type of Study**

 Research was done in Beijing, China in Peking University, a clinical research center for Oral Diseases & National Engineering Laboratory for Digital and Material Technology of Stomatology, and Beijing Key Laboratory of Digital Stomatology. This study is an example of a parallel randomized control trial where there is one experimental group and one control group.

**Study Purpose**

 The dental pulp is a vital tooth structure that is responsible for nutritional supply, dentin production, tooth sensation, and vitality maintenance. However, this structure can be easily affected by carious lesions, trauma, or idiopathic factors which can cause pulp inflammation or pulp necrosis possibly leading to an unwanted abscess. To remove the localized infection, clinical procedures, such as endodontic (root canal) therapy, are often performed. After the procedure, the tooth is devitalized and filled with synthetic biomaterials. As time passes, the tooth weakens, becomes brittle, and is prone to fractures. This leaves the tooth “useless.”

Endodontic restorative therapy sparked the author’s interest in promoting regeneration of vital pulp tissue. It is known that dental pulp stem cells (DPSCs) can promote regeneration of dental pulp tissue in tooth structure. However, these stem cells are obtained from adult patients that need to have their molars extracted. This long process delays stem cell isolation, therefore, SHEDs is a good option. SHEDs showed non-immunogenicity and a higher proliferative potential than DPSCs. Instead of devitalizing the tooth, stem cells acquired from SHEDs loaded with SMS would be an alternative method to restore the vitality of the tooth by promoting vascularized tissue regeneration. This method saves the tooth giving it another chance to properly function.

**Experimental Design**

The authors designed an injectable SIM functionalized gelatin methacrylate (GelMA) cryogel microspheres (SMS) as a medium of transport to effectively deliver SHEDs into the root canals. Many experiments were done to determine the most appropriate concentration of SIM (0μm, 0.01μm, 0.1μm, 1μm, or 10μm) and the specific microcarrier (SIM functionalized cryogel microspheres (SMS) or cryogel microspheres (CMS)). Three to five passages of SHEDs cultured with 100 microliters a-mem and other reagents (gelatin, methacrylic anhydride, 2-hydroxy-4’-(2-hydroxyethoxy-2-methylpropiophenone)) were used in the experiments and seeded on 96-well plates to create the best injectable to deliver SHEDs. This experiment was done in a span of approximately seven days.

After finding that SMS was most suitable to effectively deliver SHEDs, the complex was made and injected in vitro and in vivo to see the angiogenic and odontogenic differentiation of SHEDs. 12 SHEDs seeded in the wells each were cultured and used to see in vitro angiogenic and odontogenic potential of SHEDs. This portion took approximately seven days each. Balb/C nude mice (male, 6 weeks old, 25g average body weight) were decalcified in 17% ethylenediamine tetraacetic acid (EDTA) solution and dehydrated with gradient ethanol used as subcutaneous injection models and freshly extracted single rooted premolars were collected from healthy patients ages 15 to 30 years old in the Oral and Maxillofacial Surgery Clinic at Peking University School and Hospital of Stomatology with informed consent and institutional review board approval for in vivo SHEDs loaded SMS tooth segments implantation. The roots were obtained from the teeth and soaked in 17%EDTA, then 19% citric acid to remove any residual soft tissues. They were then sterilized and incubated for three to seven days to remove residual disinfecting agents. Afterwards, subcutaneous pockets were created on the nude mice, the root segments injected with SHEDs suspension and SHEDs/SMS were implanted, and the incision from the mice were closed with a suture. The control group was injected with the SHEDs suspension and the experimental group was injected with the SHEDs/SMS suspension. The independent variable is the SMS, and the dependent variable is the effect of SMS on tissue regeneration and biocompatibility. Samples were retrieved after 1 month and the animals were sacrificed.

When analyzing the results on effect of SMS on tissue regeneration and biocompatibility, the researchers used various staining methods (immunohistochemical, ARS, H&E, etc) to better view SHEDs suspension and SHEDs/SMS under a light microscope. All quantitive data were expressed as mean +/- standard deviation for n ≥ 3. Statistical analysis was carried out using one-way/two-way analysis of variance (ANOVA) with Turkey’s test. Differences between groups of \*p < 0.05 were considered statistically significant, \*\*p < 0.01 and \*\*\*p 0.001 were considered highly significant. Calibration was not mentioned in the journal article, and is therefore unknown whether the researchers were calibrated or not.

**Results**

* The highest absorbance values were found in the 0.01μm SIM group after 5 days of culture
* 0.01μm SIM significantly enhanced SHEDs mineralized deposit formation
	+ Statistically significant (p < 0.05)
* SHEDs stimulated by 0.01μm SIM showed the highest angiogenic gene and chemokine genes
* With increasing SIM concentration, the expression levels of angiogenic and chemokine gene in SHEDs decreased gradually
* SMS is a more suitable microcarrier compared to CMS due to its enhanced adhesion, proliferation, and cell production properties
* Sustained release of SIM in SMS promoted SHEDs angiogenic potential and remarkably enhanced angiogenesis
	+ Highly significant (p < 0.001)
* Subcutaneous results indicated that sustained release of SIM from SMS would promote SHEDs odontogenic and angiogenic potential and the SHEDs/SMS constructs would be capable of regenerating highly organized vascularized tissue
* The implantation of SHEDs/SMS injected tooth segments caused no inflammatory response in liver, kidney, and spleen of nude mice which indicated biocompatibility of engraftments
* Higher amounts of collagenous vascularized connective tissues were formed in SHEDs/SMS group suggesting that it would be able to regenerate vascularized pulp-like tissue in tooth root canals

**Conclusions**

 The authors concluded that dental derived stem cells play a role in promoting pulp regeneration but are limited when it comes to differentiating into the desired phenotype in the desired root canals. Therefore, 0.01μm SIM was used for odontogenic differentiation and angiogenic potential. At this concentration, SIM also promoted SHEDs proliferation, adhesion, and cell protection during the injection of the SIM/SHEDs complex. The SHEDs loaded with SMS showed odontogenic and proangiogenic potential in vitro and vascularized pulp-like tissue in vivo.

These findings can contribute to promising applications in endodontic regenerative dentistry. However, the authors brought attention to possible residual bacteria in the dentinal tubules during clinical trials that can lead to inflammation and later progress to pulp necrosis. SIM demonstrated anti-inflammatory properties but should be further investigated with its effect on SHEDs. In future work, the complex should be injected into the full length of the root canals and sealed to explore regenerative capacity in vivo and on larger animals with similar anatomy, physiology, pathology, and histology to humans to see if similar results can be retrieved.

As the data obtained are still part of an ongoing study, the exact methods to reproduce the findings cannot be shared at this time according to the authors

**My Impression**

This study shows a new kind of technology that can be introduced and applied well to the field of dentistry and dental hygiene. The method of stem cells’ ability to regenerate pulp would not only save the tooth, but also save the patient from possibly having to adjust to a crown. The occlusion would have to be adjusted so the patient’s bite and/or adjacent teeth are not affected. If the adjacent tooth of the crown needs a filling or any other restorative treatment, the dentist would would have to be adjust it to the crown initially placed. To retain the crown and keep it in good shape, it is imperative for the patient to have a good oral homecare routine which includes brushing thoroughly and flossing underneath the crown to remove biofilm or debris that resides. Although, technology has advanced and the crowns are similar to natural teeth, nothing can compare to ones’ natural functioning teeth.

After reading this journal article I became more intrigued with the topic than I already was and developed many curiosities. One of them being, the duration of when the dental pulp will be fully functional when regenerated from the dental stem cells. Can the tooth still be used while in treatment? Or should it be avoided at all costs because a tooth without a dental pulp is brittle and can easily fracture. I would also like to know about the number of times a tooth can go through this procedure. For example, if a tooth already regenerated dental pulp, will the stem cells work on the same tooth and be able to regenerate the pulp again with all the same abilities if the pulp somehow ends up being infected again? The questions and things to learn from this topic are endless because this would change the view and methods of dentistry.

**Reference**

Yuan, X., Yuan, Z., Wang, Y. et al. (2022). Vascularized pulp regeneration via injecting simvastatin functionalized GelMA cryogel microspheres loaded with stem cells from human exfoliated deciduous teeth. *Materials Today Bio*, 13(100209). https://doi.org/10.1016/j.mtbio.2022.100209